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Original Article

Atrophy of non-locomotor muscle in patients with end-stage renal failure

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Abstract

Background. All previous histological studies of skeletal muscles of patients with renal failure have used locomotor muscle biopsies. It is thus unclear to what degree the observed abnormalities are due to the uraemic state and how much is due to disuse. The present study was undertaken to attempt to investigate this question by examining a non-locomotor muscle (rectus abdominis) in patients with end-stage renal failure.

Methods. Biopsies from rectus abdominis were obtained from 22 renal failure patients (RFPs) undergoing surgical Tenckhoff catheter implantation for peritoneal dialysis and 20 control subjects undergoing elective abdominal surgery. Histochemical staining of frozen sections and morphometric analysis was used to estimate the proportion of each fibre type, muscle fibre area and capillary density. Myosin heavy chain composition was examined by SDS–PAGE.

Results. There were no differences in fibre type distribution between RFPs and controls. All RFPs showed fibre atrophy [mean cross-sectional area (CSA) $3300 \pm 1100 \mu\text{m}^2$, compared to $4100 \pm 1100 \mu\text{m}^2$ in controls ($P < 0.05$)]. All fibre types were smaller in mean CSA in RFPs than in controls (15, 26 and 28% for types I, IIa and IIx, respectively). These differences could not be accounted for by differences in age, gender or cardiovascular or diabetic comorbidity. Muscle fibre capillarization, expressed as capillaries per fibre

or capillary contacts per fibre, was significantly less in RFPs.

Conclusions. Since a non-locomotor muscle was examined, the effects of disuse as a cause of atrophy have been minimized. It is likely, therefore, that the decreased muscle fibre CSA and capillary density of RFPs compared to controls were due predominantly to uraemia itself.

Keywords: atrophy; non-locomotor muscle; renal failure

Introduction

Muscle wasting and weakness are common in chronic and end-stage renal failure and are associated with physiological impairments and limitations in activities of daily living [1]. An interrelated set of causal factors in these circumstances includes decreased protein/calorie intake, disuse atrophy and disordered muscle protein balance that favours catabolism [2]. In addition, anaemia and neuropathy (uraemic or diabetic) may contribute to functional impairment [3].

All previous studies of skeletal muscle morphology in renal failure have examined upper or lower limb (i.e. locomotor) muscle biopsies [4–10]. It is possible that the abnormalities observed may have been due both to the effects of uraemia and to disuse atrophy. The purpose of the present study, therefore, was to characterize the degree of abnormality found in a non-locomotor muscle of patients with renal failure, thereby largely eliminating the potential influence of disuse atrophy. It was hypothesized that the histochemical and morphometric profile of the renal failure muscle would be abnormal in comparison with age-matched controls free of renal failure.

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Subjects and methods

Patients

The renal failure patient (RFP) group consisted of 22 pre-dialysis patients (12 women and 10 men), aged 54.7 ± 14.3 (mean \pm SD) years. Patients who agreed to take part were recruited sequentially. They had reached end-stage renal failure (serum creatinine 6.4 ± 2.1 mg/dl, blood urea nitrogen 14.3 ± 4.5 mg/dl) and were studied at the time of catheter insertion for peritoneal dialysis, just prior to the institution of dialysis. The primary causes of renal failure were renovascular disease in five cases, reflux nephropathy in four, diabetic nephropathy in three, small kidneys of unknown cause in three, glomerulonephritis in three, and amyloid in one. Twelve patients had no comorbidity, eight had cardiovascular comorbidity (ischaemic heart disease, peripheral vascular disease, left ventricular dysfunction) and five were diabetic. The nutritional status of the patients was assessed by the subjective global assessment (SGA) method, using a seven-point scale [11]. The SGA involved a careful history and clinical examination. The accuracy, reproducibility and validity of this method have previously been shown in dialysis patients [12]. Blood samples were taken for measurement of creatinine, albumin concentration, haemoglobin and parathyroid hormone.

The control (CON) group consisted of 20 subjects (10 women and 10 men), aged 58.5 ± 16.9 (mean \pm SD) years, undergoing elective abdominal surgery for various conditions such as hernia repair and elective cholecystectomy, and none had infective, malignant or inflammatory conditions. Blood samples taken as a routine hospital procedure were analysed for creatinine, albumin and haemoglobin.

All subjects were given both an oral and written explanation of the purpose and procedures of the study prior to participation and gave written informed consent. All procedures were approved by the Local Research Ethics Committee.

Muscle biopsy

A biopsy (200–500 mg wet tissue) of rectus abdominis muscle was obtained from each RFP undergoing Tenckhoff catheter insertion for peritoneal dialysis [13] and from each control subject undergoing abdominal surgery. The biopsy specimen was kept moist on a piece of gauze that was moistened with normal saline. The specimen was cut into two parts at right angles in relation to the direction of muscle fibres; one part was used for histological and histochemical analysis and the remainder for determination of myosin heavy chain (MyHC) composition by gel electrophoresis.

Histological and histochemical analysis

The biopsy materials were rapidly frozen in isopentane (-155°C) for 10 s and stored in liquid nitrogen (-196°C). Serial sections of the samples were obtained by cutting transverse sections of the biopsy (10 μm thickness in a cryostat Leica CM1800, Germany) at -25°C and collected on glass slides treated with poly-L-lysine. All sections were stained on the same day of cutting, with a time delay of ~ 2 h.

A minimum of three serial sections per patient were used for histological examination; the morphological examination was

made using haematoxylin and eosin, Harris's haematoxylin. To determine the percentage of types I, IIa and IIx fibres, acid-labile myofibrillar ATPase was used [14], with pre-incubation at pH 4.40 and 4.75. For identifying the muscle fibre capillaries, an α -amylase-PAS stain was employed [15].

The morphometric analysis was performed with a camera (E.A.S.Y. 429 K) connected to a light-microscope (Zeiss axioskop) and digitizer (Mitsubishi, Video Copy Processor, Herolab: molecular technique software) connected to a PC. The cross-sectional area (CSA) of the muscle fibres from each sample was determined, the fibre type distribution was determined from sections stained for myofibrillar ATPase and the number of fibres per square millimetre was calculated from the sections stained by the haematoxylin method. A minimum number of 100 fibres was studied from each sample. Morphometric analysis was performed without prior knowledge of the group origin of the biopsy. Atrophic fibres were classified as all those fibres with a CSA of $< 50\%$ of the control mean CSA, which was specific for each fibre type: $\leq 1729 \mu\text{m}^2$ for type I, $\leq 2545 \mu\text{m}^2$ for type IIa and $\leq 1749 \mu\text{m}^2$ for type IIx.

Capillary profile

Capillaries were quantified manually from photographs taken from the previously described image analysis system. An area containing at least 150 fibres in each section was selected for capillary counting. Analysis of capillary profiles included the capillary-to-fibre ratio (C:F), i.e. the ratio of the number of capillaries occurring with 100 fibres; capillary density per square millimetre of muscle tissue (CD/mm^2); and capillary contact per fibre (CC/F), i.e. the number of capillaries that were adjacent to a single fibre and thus used as a measure of aerobic potential of skeletal muscle. All the capillary measurements were determined from at least 100 fibres, except CD/mm^2 , which included all the fibres within a specified area (mm^2). All transversely cut capillaries were counted; if a capillary was sectioned longitudinally, it was counted as one each time it crossed a junction between three or more muscle fibres.

MyHC analysis: one-dimensional gel electrophoresis

The proportion of MyHC isoforms present in the whole muscle sample was determined as follows. Muscle specimens (30–50 mg) were homogenized (1 mg of muscle tissue per 10 μl of buffer solution) in a buffer solution composed of 10% glycerol (w/v), 5% 2-mercaptoethanol (w/v), 2.3% SDS (w/v), 62.5 mM Tris and 0.001% bromophenol blue (w/v), pH 6.8 (corrected by HCl), and incubated at 80°C for 10 min. Samples were then centrifuged at 12 000 g at 4°C for 5 min. The supernatant fractions were collected and stored separately (-80°C) and later analysed by SDS-PAGE.

Vertical PAGE (0.75 mm thickness) in the presence of SDS was performed according to the method of Talmadge and Roy [16]. MyHCs were analysed in high-glycerol-containing gels (30%). MyHC isoforms from the rectus abdominis muscle were resolved into three separate bands, MyHC I and IIa and IIx, in 8 and 4% polyacrylamide for separating and stacking gels, respectively. The ratio of acrylamide and bisacrylamide in stock was 50:1. The gels were run for ~ 22 h at a constant current of 20 mA and at temperature of 4°C . The relative content of MyHC isoforms was determined by densitometry using gels stained with Coomassie blue (R250). The protein

bands were identified according to their apparent molecular masses compared to the migration of high-molecular-weight protein markers (C 3312, Sigma, Poole, UK). The densitometric system used Herolab software as described above, and the coefficient of variation of the gels analysis was 4.87%.

Statistical analysis

All analyses were carried out by using the statistical package SPSS 9.0 for Windows (SPSS, Chicago, IL). Standard descriptive statistics, consisting of means \pm SDs, were used to characterize the subject population. χ^2 analyses were used to assess percentage fibre type and MyHC distribution between the two groups. Two-way [group (CON and RFP) by fibre type (I, IIa and IIx)] mixed-model repeated measures ANOVAs were used to examine differences in CSA. Tukey HSD *post hoc* analysis was performed to indicate differences within analyses. χ^2 analysis was used to compare the percentage of atrophied types I, IIa and IIx fibres between the RFP and CON groups. Independent *t*-tests were used to examine differences in mean fibre CSA, CD/mm², CC/F and C:F between the two groups. An independent Student's *t*-test was performed to analyse the differences between malnourished and well-nourished patients as well as the clinical assessment data. An α level of $P \leq 0.05$ was selected to indicate statistical significance.

Results

Haematological and biochemical evaluation

The RFP group showed significantly higher serum creatinine concentration ($t_{40} = 11.8$, $P < 0.01$) and lower albumin ($t_{40} = -2.8$, $P < 0.01$) and haemoglobin concentration ($t_{40} = -9.4$, $P < 0.01$) than the CON group (Table 1).

Muscle fibre type composition: histochemical analysis

In the rectus abdominis muscle of the CON group, the dominant fibre types were types I and IIa, which together accounted for 88% of all fibres present, and 12% of the fibres were identified as type IIx (Table 2).

A similar pattern was found in the RFP group, in which types I, IIa and IIx fibres accounted for 49, 43 and 8%, respectively. χ^2 analyses indicated that no significant differences existed between the groups when comparing the fibre type distribution ($P > 0.05$) or

percentage distribution of MyHC isoforms ($P > 0.05$) (Table 2).

MyHC isoform composition: SDS-PAGE

Similar to the histochemical analysis of fibre type composition, the dominant MyHC isoforms were types I and IIa, which accounted for 90% of the total MyHC in both groups. χ^2 analysis revealed no statistically significant differences between the groups concerning the three different types of MyHC isoforms ($P > 0.05$) (Table 2).

Muscle fibre CSA

Type I fibres were revealed to be significantly smaller in CSA than type IIa fibres ($F_{2,39} = 19.654$, $P < 0.05$). This corresponded to a 32 and 22% smaller CSA for the CON and RFP groups, respectively (Figure 1). Type IIx fibres, which accounted for 5–7% of all fibres, also had a smaller mean CSA compared to type IIa in both groups ($F_{2,39} = 19.654$, $P < 0.05$) (Figure 1). All RFPs showed a general muscle fibre atrophy, and the mean CSA was 21% smaller ($3300 \pm 1100 \mu\text{m}^2$) than in the CON group ($4100 \pm 1100 \mu\text{m}^2$, $F_{1,43} = 7.5$, $P < 0.05$); hence, the number of fibres per square millimetre of muscle was higher (288 ± 54 vs 254 ± 78 , $F_{1,43} = 2.069$, $P = 0.05$) in the RFP group than in the CON group. The fibre population most affected in the RFP group was the type IIa fibres, which were 26% smaller than in the CON group (3800 ± 1600 vs $5100 \pm 1500 \mu\text{m}^2$), and the type IIx fibre CSA was 28% smaller than in the CON group (2500 ± 1700 vs $3500 \pm 1200 \mu\text{m}^2$, $F_{1,40} = 7.5$, $P < 0.05$) (Figure 1). Additional analysis of the renal failure data demonstrated that those patients with some form of comorbidity had smaller type I fibres than those with renal failure alone (2518 ± 786 vs $2892 \pm 1120 \mu\text{m}^2$, $P = 0.029$). There were, however, no differences in the size of the other types of muscle fibres between RFPs with and without some form of comorbidity or diabetes (Table 5).

Nutritional status

The SGA showed that seven of the 22 RFPs were malnourished (B or B⁺). No significant differences in

Table 1. Haematological and biochemical variables in the renal failure patient (RFP) and control (CON) groups

	RFP	CON
Creatinine (60–120 $\mu\text{mol/l}$)	567 ± 187^a	92 ± 25
Albumin (35–50 g/dl)	37 ± 2^a	42 ± 6
Haemoglobin (13–18 g/dl)	10 ± 1^a	14 ± 1
Mean venous TCO ₂ (24–32 mmol/l)	22 ± 3	–
Parathyroid hormone (6–20 pmol/l)	19 ± 13	–

^aSignificant differences between groups ($P < 0.01$). TCO₂, total bicarbonate.

Table 2. Fibre type distribution (%) and relative myosin heavy chain (MyHC) content (%) in the renal failure patient (RFP, $n = 22$) and control (CON, $n = 20$) groups based on histochemical and SDS-PAGE analyses

	RFP	CON
Fibre type		
Type I	49 ± 9	50 ± 14
Type IIa	43 ± 11	38 ± 14
Type IIx	8 ± 8	12 ± 12
MyHC content		
MyHC I	48.6 ± 11.9	47.3 ± 14.9
MyHC IIa	40.6 ± 14.1	42.6 ± 14.5
MyHC IIx	10.8 ± 13.2	10.1 ± 10.9

Values are presented as means \pm SD.

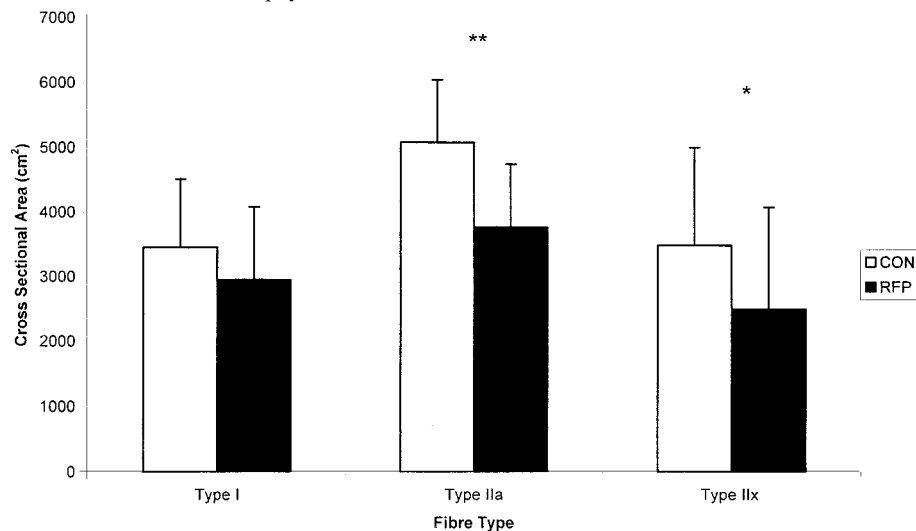


Fig. 1. Fibre type cross-sectional area (CSA, mean \pm SD) for renal failure patients (open bars; $n = 22$) and controls (closed bars; $n = 20$). Statistical significant differences in fibre size between groups were found for type IIa (** $P < 0.01$) and IIx fibres and mean CSA (* $P < 0.05$).

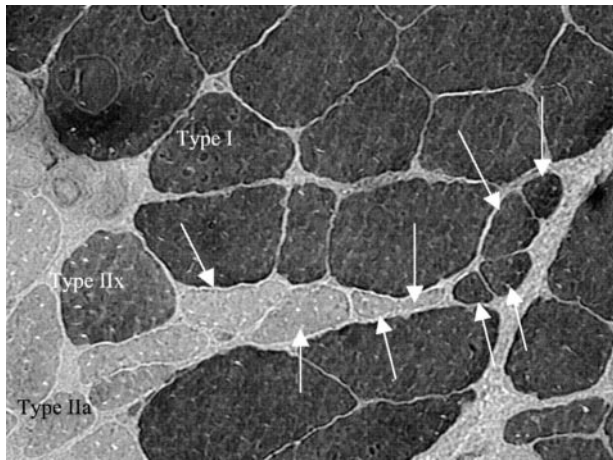


Fig. 2. Photomicrograph of renal failure patient muscle biopsy, showing muscle fibre atrophy and random distribution in fibre size and shape.

muscle fibre CSA were observed between malnourished and well-nourished patients ($P > 0.05$).

Atrophy

Compared with the CON group, the muscle biopsies of the rectus abdominis muscle from the RFP group showed three times as many atrophied muscle fibres with random distribution in fibre size and shape within the muscle (Figure 2). In the CON group, only 10% of all fibres were classified as atrophic; in comparison, in the RFP group, 27% of all fibres have been calculated as being atrophic ($\chi^2 = 42.55$, $P < 0.05$) (Table 3). In the type II fibre populations of the RFP group, 36% of the fibres were atrophic. In the same fibre population of the CON group, the atrophy was nearer to 12% ($\chi^2 = 41.33$, $P < 0.05$). Atrophied fibres were found both in isolation and in small groups of

Table 3. The percentage of atrophic fibres in the renal failure patient (RFP, $n = 22$) and control (CON, $n = 20$) groups

	RFP	CON
Total	27.0 ^a	10.0
Type I	15.5	6.6
Type IIa	33.4 ^a	12.3
Type IIx	46.8 ^a	10.8
Type II	36.0 ^a	12.0

The total percentage value represents the number of atrophic fibres in RFP and CON groups independent of fibre type. The different fibre type percentage values represent the number of atrophic fibres within each specific fibre type population.

^aSignificant differences ($P < 0.05$).

2–5 fibres together; these were surrounded by relatively normal-sized fibres and occasionally adjacent to unusually large hypertrophied fibres (Figure 3).

Muscle fibre capillarization

The CON group had 20% greater capillary density than the RFP group when expressed as capillaries per fibre ($t_{41} = -5.7$, $P < 0.05$) but not when expressed as CD/mm² of muscle ($P > 0.05$); as described above, this difference is due to the higher number of fibres per square millimetre of muscle. The CON group also had a greater CC/F than the RFP group ($t_{41} = -3.8$, $P < 0.05$) (Table 4).

Centronucleation

The nuclei of muscle fibres are usually arranged around the periphery of the fibres; nuclei that are located within the fibre are usually indicative of a myopathy or dystrophic process. The proportion of fibres with central located nuclei was $5 \pm 4\%$ of the total fibre number in both groups.

Other histological abnormalities

The biopsies of RFPs sometimes showed clumping of fibre types and a loss of normal mosaic pattern of fibre type distribution (Figure 4).

Other abnormal features, such as large fibres with different shapes and clusters of fibres undergoing phagocytosis, were observed.

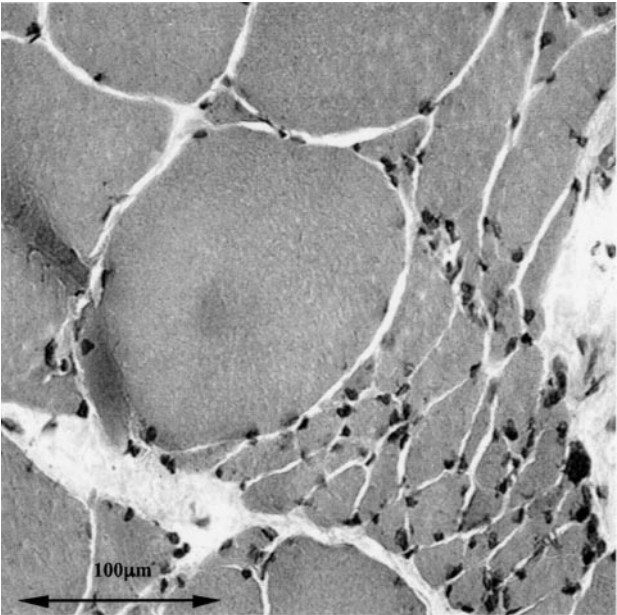


Fig. 3. Photomicrograph of renal failure patient muscle biopsy, showing groups of atrophied fibres surrounded by relatively normal-sized fibres.

Table 4. Capillarization of muscle and the number of capillary contacts per muscle fibre and capillaries per muscle fibre in the renal failure patient (RFP) and control (CON) groups

Capillary factors	RFP	CON
CD/mm ²	320 ± 60	340 ± 90
CC/F	3.0 ± 0.4	4.0 ± 0.5 ^a
C:F	1.12 ± 0.14	1.35 ± 0.15 ^a

CD/mm², capillary density per square millimetre of muscle tissue; CC/F, capillary contacts per muscle fibre; C:F, capillary-to-fibre ratio.

^aSignificant differences ($P < 0.01$).

Discussion

This is the first study of the morphology and morphometry of rectus abdominis muscle biopsies in pre-dialysis patients in end-stage renal failure. The only other study of rectus abdominis in RFPs, by Conjard *et al.* [17], did not include histological data. Muscle fibre type distribution and MyHC content in RFP biopsies were not different from control subjects and are similar to those previously reported by Haggmark and Thorstensson [18] for abdominal wall muscles of normal control subjects. Patient samples showed significant generalized muscle fibre atrophy, most marked in types IIa and IIx fibres (25–30% smaller than controls), such that the number of fibres per square millimetre of muscle was greater in RFPs. Muscle fibre capillarization, expressed as capillaries per muscle fibre, was significantly reduced compared to controls. Additional abnormal histological features of note were a wide range of fibre sizes, fibre type clumping and some necrotic fibres with associated inflammatory cell infiltrate.

In the only published study of pre-dialysis patients, Clyne *et al.* [1] found, as in the present study, generalized muscle fibre atrophy with a greater degree of atrophy in type II than in type I fibres. In contrast, though, they observed a greater proportion of type I fibres in the patients' muscle. Since they studied biopsies of vastus lateralis, this discrepancy in fibre type distribution may be due to the confounding affect associated with disuse atrophy, resulting from reduced locomotory activity in the patients they studied. Most other studies of skeletal muscle morphology in renal failure have exclusively examined the locomotor muscle group, the quadriceps, in haemodialysis patients [2,4–10]. Common findings in these studies, as in the present study, were of fibre atrophy, mostly of type IIa fibres, variation in fibre size and fibre type grouping. Only two other studies have investigated a semi-non-locomotor muscle, the deltoid. Both were conducted in haemodialysis patients, one in adults [9], one in children [10]. The deltoid may be considered to be intermediate between rectus abdominis and quadriceps muscles in terms of involvement in locomotion and therefore subject to the effects of disuse atrophy. Nevertheless, the findings in these two studies were broadly similar to the present study, demonstrating type II fibre atrophy, fibre size variation and fibre type grouping. These

Table 5. Muscle fibre morphometry of the five renal failure patients whose comorbidity was diabetes

Type I (µm)	Type IIa (µm)	Type IIx (µm)	Type I (%)	Type IIa (%)	Type IIx (%)	CD/mm ²	CC/F	C:F
3000	3700	1500	66	32	2	280	3.4	1.10
1800	1300	1100	35	59	6	400	2.7	1.14
1900	1900	1500	52	46	2	341	2.9	0.97
1500	3200	2100	48	47	5	451	3.3	1.32
2500	3400	3600	59	40	1	330	3.0	1.10

CD/mm², capillary density per square millimetre of muscle tissue; CC/F, capillary contacts per muscle fibre; C:F, capillary-to-fibre ratio.

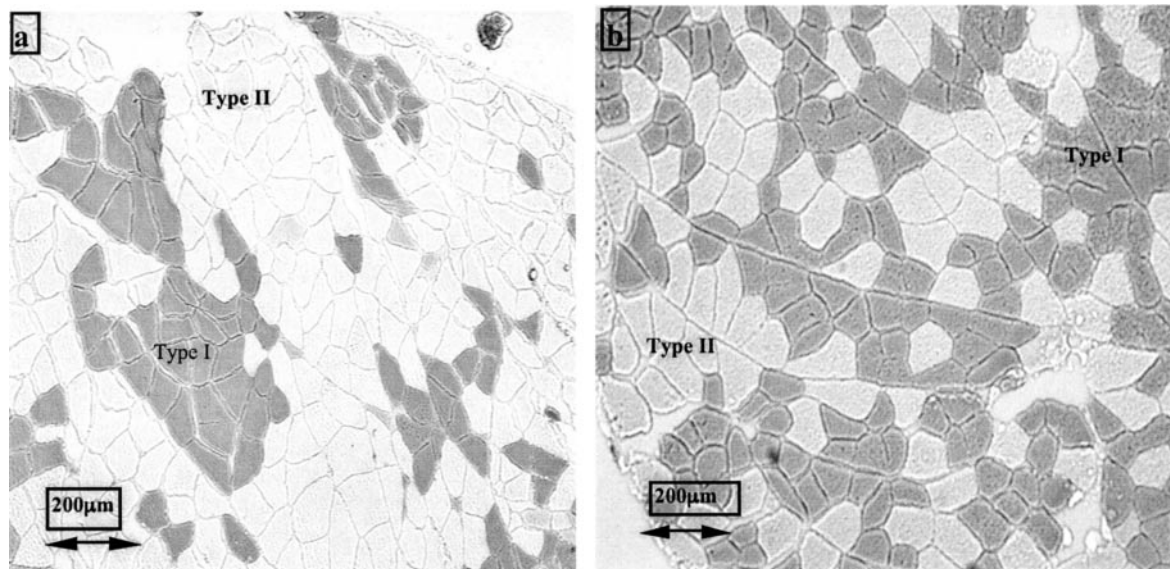


Fig. 4. Photomicrographs of renal failure patient muscle biopsy, showing clusters of fibres undergoing phagocytosis.

similarities are, perhaps, all the more remarkable, since the patients of Bautista *et al.* [9] were reported as showing 'proximal paresis'.

The likely systemic factors that may be associated with fibre atrophy in the RFPs in the present study include metabolic acidosis, peripheral neuropathy, malnutrition, anaemia or diabetic or cardiovascular comorbidity. Since the controls and RFPs were matched for age and gender, these potential influences on muscle fibre CSA cannot have accounted for the differences between the groups. Acidosis has been shown to be associated with increased muscle protein catabolism in patients with chronic renal failure [19]. There are no published muscle biopsy studies of the effects of acidosis correction in renal failure. Although this intervention has been shown to decrease amino-acid catabolism in chronic renal failure [20], neither of the two clinical studies of acidosis correction in dialysis patients was able to document an increase in muscle mass or nutrition [21,22]. Our patients were mildly acidotic, and therefore we cannot rule out a catabolic effect from having a mild metabolic acidosis.

Peripheral neuropathy is associated with fibre atrophy and the changes in fibre type grouping [23] similar in pattern to that of other investigators and our observations in RFP muscle. None of our patients exhibited overt signs or symptoms of neuropathy. Our findings may indicate, however, that sub-clinical neuropathy is more widespread than is currently appreciated. Fibre atrophy following denervation is, like that associated with acidosis, mediated by the ubiquitin/proteasome system, but no experimental studies investigating the combined influence of a combination of renal failure and denervation have been published.

Malnutrition and starvation have been shown to be associated with muscle fibre wasting in a number of clinical and experimental settings [7]. A decrease in protein/calorie intake is known to occur early in the

progression of chronic renal failure, and malnutrition is common in the pre-dialysis population. Fahal *et al.* [7] found a significant difference in fibre CSA in quadriceps biopsy samples between adequately nourished and malnourished haemodialysis patients. In contrast, we did not find any differences in mean muscle CSA between subjects categorized as adequately nourished or malnourished, as defined by the seven-point SGA scale. They used a combination of plasma concentrations of visceral proteins and the three-point SGA grading as the means of measuring nutritional status. Thus, their categorization of malnutrition was not the same as ours. This, and any possible additional factors contributed by the process of haemodialysis, may explain the differences between the two sets of findings. Since skeletal muscle represents the major store of body protein, the degree of fibre wasting in the present study is potentially very important. It shows that up to about one-fifth loss of skeletal muscle contractile protein may exist in pre-dialysis patients. Thus, the seven-point SGA scale, a commonly used clinical nutrition measurement tool, may underestimate the degree of malnutrition in this patient population. It is possible that the lower serum albumin level in the RFPs compared with the controls was in part a reflection of malnutrition.

It is possible that anaemia may have had a role in determining fibre atrophy. This proposal is based on the findings of Davenport *et al.* [8], who found that treatment of anaemia with erythropoietin was associated with an increase in muscle type I fibre CSA in a group of haemodialysis patients. Since the main abnormality in the RFP biopsies related to type II fibres, it seems unlikely that anaemia was an influence. The mechanism of their observations is not clear, although increased oxygen delivery or increased physical activity may be involved. In line with these observations, it has been reported there is an increase in muscle strength following erythropoietin treatment [24].

Bed-rest and limb immobilization are associated with marked muscle fibre wasting in periods as short as 4 weeks, with type II fibres being particularly affected, together with a decrease in oxidative enzyme content [25]. The appearances associated with bed-rest and those seen in locomotor muscle biopsies in RFPs are, therefore, quite similar. The fact that Kouidi *et al.* [2] showed that fibre atrophy was decreased following a period of exercise training supports a proposal that disuse contributes to this abnormality in locomotor muscles in RFPs. Rectus abdominis is not directly a locomotor muscle, although it has a role as a trunk stabilizer during movement. It is unlikely, therefore, that disuse atrophy is a significant determinant of the fibre atrophy observed in our patient samples.

The finding that mean muscle fibre CSA was not different between those RFPs with and those without cardiovascular or other comorbidity, and that no differences in type II fibre size was found, indicate that these comorbidities are unlikely to have accounted for the fibre size reduction and atrophy in RFPs.

Our observation of decreased C:F ratio in RFPs is novel. Disuse atrophy induced by bed-rest or limb immobilization is associated with a decrease in capillary density [26]. Moore *et al.* [6] observed a low C:F ratio in quadriceps biopsy samples of haemodialysis patients, which was not significantly improved by the exercise intervention they used. Bradley *et al.* [4] and Kouidi *et al.* [2] reported muscle capillary abnormalities, seen on electron microscopy, in haemodialysis patients. Kouidi *et al.* [2] also reported an improvement in capillary density following their exercise intervention trial, but they did not provide any numerical data of capillary density. It is likely, however, that physical activity status explains some of the changes seen in lower limb muscle biopsies in RFPs. As discussed above, this influence is likely to be much less relevant in the abdominal muscle investigated in the present study. It may be of significance that a similar defect in fibre capillarization has been observed in myocardial tissue of dialysis patients [27]. It is possible that these findings and those of Bradley *et al.* [4], Moore *et al.* [6] and Kouidi *et al.* [2], taken with ours, indicate that a systemic defect of angiogenesis may exist in renal failure.

The results of studies by previous workers suggest that it is likely that disuse atrophy contributes to the findings of degenerative changes in locomotor muscles in RFPs. There are, however, many features common to those we report in rectus abdominis, a muscle little involved in locomotion. In addition, these features are present in dialysis and in pre-dialysis patients.

We conclude that the state of renal failure itself ('uraemia') plays an important role in the reduction of the capillary density as well as the muscle size in pre-dialysis patients. These abnormalities in skeletal muscle of patients with end-stage renal disease have at least two important implications: (i) an even greater degree of malnutrition may exist in pre-dialysis patients than is defined by a commonly used clinical measure; and (ii) exercise training interventions aimed at increasing

muscle bulk may need to be considered as a means of improving not only physical function, but also nutrition, in the renal failure population.

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Conflict of interest statement. None declared.

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